Low-Dose D-Methionine and N-Acetyl-L-Cysteine for Protection from Permanent Noise-Induced Hearing Loss in Chinchillas
Royce E. Clifford, John K. M. Coleman, Ben J. Balough, Jianzhong Liu, Richard D. Kopke and Ronald L. Jackson

Otolaryngology -- Head and Neck Surgery 2011 145: 999 originally published online 12 July 2011
DOI: 10.1177/0194599811414496

The online version of this article can be found at:
http://oto.sagepub.com/content/145/6/999

Published by:
SAGE
http://www.sagepublications.com

On behalf of:
AMERICAN ACADEMY OF OTOLARYNGOLOGY--HEAD AND NECK SURGERY
American Academy of Otolaryngology- Head and Neck Surgery

Additional services and information for Otolaryngology -- Head and Neck Surgery can be found at:
Email Alerts: http://oto.sagepub.com/cgi/alerts
Subscriptions: http://oto.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav

Version of Record - Nov 30, 2011
OnlineFirst Version of Record - Jul 12, 2011
What is This?
Low-Dose D-Methionine and N-Acetyl-L-Cysteine for Protection from Permanent Noise-Induced Hearing Loss in Chinchillas

Royce E. Clifford, MD, MPH1, John K. M. Coleman, PhD1, Ben J. Balough, MD1, Jianzhong Liu, PhD1, Richard D. Kopke, MD2,3,4, and Ronald L. Jackson, PhD1

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. Despite efforts at public health awareness and stringent industrial standards for hearing protection, noise-induced hearing loss (NIHL) remains a formidable public health concern. Although many antioxidants have proven to be beneficial in the laboratory for prevention of permanent NIHL, low-dose combinations of compounds with different biochemical mechanisms of action may allow long-term administration with fewer side effects and equal efficacy. The mixture of D-methionine and N-acetyl-L-cysteine administered at levels less than 10% of standard dosing has not been previously reported.

Study Design. Twenty-six female adult Chinchilla laniger were placed in 4 study groups, consisting of (1) a group receiving combination 12.5 mg/kg each D-methionine and N-acetyl-L-cysteine (DMET/NAC group), (2) a group receiving 12.5 mg/kg D-methionine (DMET-only group), (3) a group receiving 12.5 mg/kg N-acetyl-L-cysteine (NAC-only group), and (4) saline controls.

Setting. Laboratory.

Subjects and Methods. All groups received twice-daily intraperitoneal injections 2 days prior to noise exposure, 1 hour before and after exposure on day 3, and for 2 days subsequently, totaling 10 doses of 125 mg/kg for each antioxidant over 5 days.

Results. Although NAC-only animals paralleled saline control recovery during 3 weeks, the DMET-only group revealed gradual improvement with statistically significant recovery in the middle frequencies. The DMET/NAC group showed significant improvement at most frequencies compared with controls (P < .001 and P < .05).

Conclusion. Significant recovery of hearing was observed following continuous noise exposure with either DMET only or a combination of low-dose DMET/NAC, demonstrating a considerably lower dose of antioxidants required than previously reported for hearing recovery following acoustic trauma.

Keywords

D-methionine, N-acetyl-L-cysteine, noise-induced hearing loss, low-dose combination, chinchilla

Received April 3, 2011; accepted June 1, 2011

D espite efforts at public health awareness and stringent industrial standards for hearing protection, noise-induced hearing loss remains a major public health concern. The National Institute on Deafness and Other Communication Disorders estimates that 15% of Americans between the ages of 20 and 69 years currently have hearing loss caused by exposure to loud noise.1 The National Institute for Occupational Safety and Health reports that 20 million active target shooters in the United States are at risk (this number refers to people who have nonoccupational exposure to firearm noise and does not include the 1 million local, state, and federal police officers who regularly discharge firearms).2 In the military, hearing loss and tinnitus are the most common service-connected disabilities, with more than 445,000 veterans receiving

1Department of Otolaryngology—Head and Neck Surgery, Naval Medical Center, San Diego, California, USA
2Hough Ear Institute, Oklahoma City, Oklahoma, USA
3University of Oklahoma Health Sciences Center, Departments of Physiology and Otolaryngology, Oklahoma City, Oklahoma, USA
4Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

This article was presented as a poster at the Midwinter Conference of the Association for Research in Otolaryngology; January 2002; St. Petersburg, Florida.

Corresponding Author:
Royce E. Clifford, Department of Otolaryngology—Head and Neck Surgery, 34800 Bob Wilson Drive, Suite 200, Naval Medical Center, San Diego, CA 92134-5000
Email: royce.clifford@usmc.mil

Downloaded from otol.sagepub.com at SOCIETADE BRASILEIRA DE CIRUR on February 7, 2012
compensation for hearing loss\(^3\) due to ship noise, artillery, and exposure to improvised explosive devices.

In the laboratory, D-methionine (DMET) and N-acetyl-L-cysteine (NAC) individually have been shown to prevent permanent hearing loss and cochlear hair cell loss from acoustic trauma.\(^4-6\) Promising results have been consistent over different species, dosages, and noise exposures.\(^6\) DMET demonstrates efficacy in the range of 200 to 400 mg/kg,\(^4,5\) whereas NAC significantly reduces noise-induced hearing loss in doses from 50 to 500 mg/kg.\(^6,7\)

Combinations of antioxidants may allow administration of lower doses of individual compounds, thereby limiting toxicity or adverse side effects. Large doses of antioxidants may repress endogenous antioxidant systems; a meta-analysis of vitamins discovered increased mortality with long-term use.\(^8\) Although DMET and NAC respectively have been used as safe standard therapy in the United States and Europe for decades in the prevention of liver damage from acetaminophen overdose, morbidity and mortality have been reported with high doses or chronic use.\(^9,10\)

It is well established that cochlear outer hair cells react to damaging noise by creating reactive oxidative species (ROS) and reactive nitrate species (RNS).\(^11\) Glutathione (GSH) is a key tripeptide that reduces and conjugates ROS and RNS formed during intracellular oxidative stress. NAC then replenishes GSH by supplying a stable precursor of cysteine, cysteine being the limiting substrate in GSH production.\(^9\) DMET, in addition to being a precursor of cysteine for glutathione (GSH) production,\(^4\) acts in its own amino acid capacity as an antioxidant within functioning proteins. Evidence suggests methionine also functions in an epigenetic role by supplying methyl groups for both histone and DNA methylation (Figure 1).\(^12,13\)

Because NAC and DMET appear to operate on different aspects of the intracellular redox system in the inner ear,\(^6,14,15\) we proposed a combination with possible additive or synergistic effects, with the thought that lower dosing in combination may lead to efficacy while ensuring safety. Although mixtures of other antioxidants have been shown to be beneficial in both prevention and rescue from noise-induced hearing loss,\(^16-18\) the usefulness of NAC and DMET administered together or in these low doses has not been reported.

**Materials and Methods**

Twenty-six adult female chinchillas (*Chinchilla laniger*) were placed in 4 study groups. This species was chosen because of similarity to human cochlear anatomy and hearing range and the absence of age-related hearing loss in this species. The 4 treatment groups received intraperitoneal injections of (1) 12.5 mg/kg DMET and NAC (DMET/NAC group), (2) DMET 12.5 mg/kg (DMET-only group), (3) NAC 12.5 mg/kg (NAC-only group), or (4) identical volumes of saline. Animals received injections twice daily for 5 days, beginning 2 days prior to noise exposure. Antioxidant solutions were prepared daily prior to injection.

On the day of exposure, animals received injections 1 hour prior to and 1 hour following 6 hours of continuous noise exposure to 105 ± 0.5 dB SPL octave-band noise centered at 4 kHz, generated by a standard audiometer (GSI 16; Grason Stadler Instruments, Milford, New Hampshire) selected to white noise and routed through an attenuator (HP 350 D; Hewlett-Packard Corp, Palo Alto, California), a band-pass filter (Krohn-Hite 3550R; Krohn-Hite Corp, Avon, Massachusetts), and a power amplifier (Crown Audio Inc, Elkhart, Indiana) to an audiometric loudspeaker (JBL model 2350A; JBL Inc, Northridge, California) suspended directly above the animal’s cage. The sound spectrum output of the system was confirmed using a Larson and Davis model 800B (Larson Davis, Provo, Utah) sound level meter (A scale, SPL).\(^4\)

All 4 groups underwent auditory brainstem response (ABR) testing within 2 hours after noise exposure (week 0) and then once per week for the subsequent 3 weeks using skin ABR (SmartEP, Intelligent Hearing Systems, Miami, Florida). Subcutaneous 30-g needle electrodes were placed in the skin.
of the head and posterior to each ear. Digitally generated stimuli consisted of tone pips (4-millisecond Blackman rise/fall ramp, 0-millisecond plateau, and alternating phase) at octave intervals of 2, 4, 6, and 8 kHz. Six hundred samples were collected from the recording electrode, amplified (50,000-75,000), filtered (100-1500 Hz), and fed to an A/D converter computerized on a signal processing board. Stimuli at a rate of 23/s were varied in 10-dB descending steps until threshold was reached, and then 5-dB ascending steps were presented to confirm threshold. ABR was measured at 2000, 4000, 6000, and 8000 Hz. All reported measurements represent shifts from individual baseline values.

Analysis was performed using multiple 2-way analysis of variance (ANOVA), 2-tailed, with the Holm-Sidak test for pairwise comparisons in cases of ANOVA with \( P < .05 \). A power analysis\(^{19} \) based on a critical difference of 40% to 50% mean reduction in 21-day post–noise exposure ABR threshold shifts with a standard deviation 20% and using a 30% decrease in mean outer hair cell count with a standard deviation 20% indicated adequate sample sizes of 5 to 7 animals to achieve a \( \beta \) error of .2 to .1, respectively, with an \( \alpha \) error of .05.

The Institutional Animal Care and Use Committee of Naval Medical Center San Diego (NMCSD) approved the experimental protocol. Animals were procured, maintained, and used in accordance with the Animal Welfare Act of 1996, as amended, and the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council. Animals were housed in the NMCSD animal facility accredited by the Association for the Accreditation of Laboratory Animal Care International. No animals were sacrificed as a result of these experiments.

### Results

Baseline ABR thresholds for left and right ears obtained within 2 hours prior to noise exposure on all animals revealed no statistical difference between controls and animals subsequently injected with antioxidants at any of the frequencies, and all animals had normal ABR thresholds in all 4 frequencies (data not shown). Left and right ears were treated as individual data points, and no statistical difference between ears in any animal was noted.

Two hours after exposure, all chinchillas were noted to have threshold shifts ranging from 65 dB to approximately 100 dB across frequencies (Table 1). Initially, all treated animals sustained a temporary threshold shift an average of 6 to 7 dB higher than saline controls at 4000 and 8000 Hz (\( P < .05 \) and \( P < .001 \), respectively), and NAC-only animal thresholds were significantly higher than controls at all frequencies measured. Both DMET/NAC animals and controls improved at approximately the same rate for the first 2 weeks. Three weeks post noise exposure, DMET/NAC led to significant improvements at 2, 4, and 6 kHz (\( P < .05 \)) and to clinically significant improvement in all frequencies compared with controls.

Because this combination had proven effective in reducing the permanent threshold shift, we attempted to ascertain the input for each compound. DMET-only animals appeared to

<table>
<thead>
<tr>
<th>Table 1. Treatment vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>2000 Hz</td>
</tr>
<tr>
<td>Week 0</td>
</tr>
<tr>
<td>DMET/NAC</td>
</tr>
<tr>
<td>DMET</td>
</tr>
<tr>
<td>NAC</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Week 1</td>
</tr>
<tr>
<td>DMET/NAC</td>
</tr>
<tr>
<td>DMET</td>
</tr>
<tr>
<td>NAC</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Week 2</td>
</tr>
<tr>
<td>DMET/NAC</td>
</tr>
<tr>
<td>DMET</td>
</tr>
<tr>
<td>NAC</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Week 3</td>
</tr>
<tr>
<td>DMET/NAC</td>
</tr>
<tr>
<td>DMET</td>
</tr>
<tr>
<td>NAC</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

The column headings are auditory brainstem response (ABR) threshold shifts from baseline. Amounts in parentheses are standard deviations.

Abbreviations: DMET, D-methionine; NAC, N-acetyl-L-cysteine.

aSignificant threshold shifts compared with controls. \( \downarrow \downarrow P < .05, \downarrow \downarrow \downarrow P < .001 \).

bSignificant recovery indicated by up arrows \( \uparrow \uparrow P < .05, \uparrow \uparrow \uparrow P < .001 \).
Table 2. Comparison of Treatments

<table>
<thead>
<tr>
<th></th>
<th>2000 Hz</th>
<th>4000 Hz</th>
<th>6000 Hz</th>
<th>8000 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMET/NAC</td>
<td>65.4 (7.2)</td>
<td>92.5 (2.6)</td>
<td>97.5 (3.4)</td>
<td>92.9 (3.6)</td>
</tr>
<tr>
<td>DMET</td>
<td>70.8 (4.2)</td>
<td>89.2 (4.7)</td>
<td>97.1 (4.5)</td>
<td>93.8 (2.6)</td>
</tr>
<tr>
<td>NAC</td>
<td>↓74.2 (5.2)*</td>
<td>91.7 (4.9)</td>
<td>101.7 (3.9)</td>
<td>92 (2.0)</td>
</tr>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMET/NAC</td>
<td>24.6 (2.6)</td>
<td>43.3 (7.5)</td>
<td>53.3 (6.8)</td>
<td>51.7 (7.8)</td>
</tr>
<tr>
<td>DMET</td>
<td>22.1 (7.5)</td>
<td>↑↑31.7 (6.2)</td>
<td>49.6 (8.4)</td>
<td>47.1 (8.9)</td>
</tr>
<tr>
<td>NAC</td>
<td>↓34.2 (8.8)</td>
<td>50 (7.1)</td>
<td>↓64.2 (5.6)</td>
<td>↓↓61.7 (8.1)</td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMET/NAC</td>
<td>10.4 (4.5)</td>
<td>29.6 (25.0)</td>
<td>35.4 (9.6)</td>
<td>37.1 (7.5)</td>
</tr>
<tr>
<td>DMET</td>
<td>11.2 (14.0)</td>
<td>↑↑13.8 (11.9)</td>
<td>25.4 (16.0)</td>
<td>↑25 (14.0)</td>
</tr>
<tr>
<td>NAC</td>
<td>↓22.5 (5.4)</td>
<td>37.9 (9.9)</td>
<td>↓↓52.9 (8.4)</td>
<td>↓↓52.1 (5.8)</td>
</tr>
<tr>
<td>Week 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMET/NAC</td>
<td>↑2.5 (4.0)*</td>
<td>12.9 (9.2)</td>
<td>18.8 (6.4)</td>
<td>19.6 (8.6)</td>
</tr>
<tr>
<td>DMET</td>
<td>5.8 (6.7)</td>
<td>10.8 (11.0)</td>
<td>22.9 (11.0)</td>
<td>20.8 (9.2)</td>
</tr>
<tr>
<td>NAC</td>
<td>↓↓16.7 (3.9)</td>
<td>↓25.8 (8.8)</td>
<td>↓↓40 (8.8)</td>
<td>↓↓38.8 (8.0)</td>
</tr>
</tbody>
</table>

The column headings are auditory brainstem response (ABR) threshold shifts from baseline. Amounts in parentheses are standard deviations.

Abbreviations: DMET, D-methionine; NAC, N-acetyl-L-cysteine.

*Significant threshold shifts compared with controls (↓P < .05, ↓↓P < .001).

*Significant recovery indicated by up arrows (↑P < .05, ↑↑P < .001).

return toward baseline at a faster rate during weeks 1 and 2 than did DMET/NAC animals in the higher frequencies, after which hearing improved only slightly. At week 3, all frequencies in the DMET-only group were closer to baseline than controls. DMET-only ears achieved statistical significance only at 4 and 6 kHz (P < .001 and P < .05, respectively).

Table 2 shows a comparison of treatments, and there is seen to be no clinical difference between DMET/NAC and DMET-only groups. The differences between DMET/NAC and DMET-only groups is less than 5 dB in all frequencies.

During weeks 0 and 1, animals treated with NAC only maintained a higher threshold shift than did controls. At week 2, NAC-only animals showed less return to baseline than did DMET/NAC and DMET-only chinchillas. The NAC-only group completed the third week in the same range as control animals (Table 1) and showed significantly less improvement (P < .05) than did DMET/NAC or DMET-only animals at week 3 (Table 2).

Control animals returned toward baseline through week 2 and did not change significantly during the following week, whereas DMET/NAC animals showed gradual return toward baseline over all frequencies throughout the entire 3 weeks.

Discussion

The mechanism of action of these 2 antioxidants may assist in elucidating the ultimate minimal effective dose used in combination. It is well established that acoustic trauma and acetaminophen toxicity both lead to formation of intracellular RNS and ROS, unstable anions generated within and released from mitochondria. RNS and ROS are generated for at least 7 to 10 days after injury, both in hepatic damage and in acoustic trauma.11 In the ear, if neutralization does not occur, ROS and RNS will damage proteins, DNA, and cell membranes, leading to loss of auditory mechanosensory cells, that is, outer hair cells. In the liver, hepatic failure can ensue. Two well-described intracellular systems that clear mitochondria and cytoplasm of these reactive species are GSH and the methionine sulfoxide reductase (MSR) group of enzymes.4,20 Both systems donate sulfhydryl moieties for elimination of ROS and RNS, either in free cytoplasm and mitochondria, in the case of GSH, or for reduction of oxidized methionine amino acid residues, in the case of MSR.

N-acetyl-L-cysteine is an orally bioavailable and stable precursor to cysteine, the rate-limiting amino acid in the production of GSH, a ubiquitous tripeptide found in mitochondria, cytosol, and the nucleus.5,11 GSH donates its sulfhydryl protons for neutralization of ROS and RNS, and the resulting GS adducts are either excreted into plasma or dimerized via covalent bonding at the sulfhydryl group to create glutathione disulfide (GSSG). GSSG is then reduced by the enzyme glutathione reductase for regeneration (Figure 1) or transported to plasma for excretion.21 Replenishment of intracellular GSH is performed either by reduction of GSSG to GSH via glutathione reductase or by de novo synthesis.

Methionine is also a precursor of cysteine, via demethylation. DMET has a more stable shelf life and better bioavailability than does L-methionine22 and is rapidly changed to its L-enantiomer after plasma absorption.23 L-methionine is then involved in a biochemical redox system as part of its amino acid incorporation into the protein backbone. Methionine, the most readily oxidized of all amino acids,20 neutralizes ROS and RNS either in its protein milieu or free in the cytosol by absorbing electrons to become a methionine sulfoxide residue. This amino acid is overrepresented in mitochondrial electron transfer.
transport proteins, constituting an average of 6.17% in animal mitochondria, 3 times that of prokaryotes. Importantly, methionine constitutes a full 10% of surface amino acid residues of cytochromes B and C and subunits in complexes III and IV of the intramembranous mitochondrial oxidative phosphorylation system. At exposed sites on these electron transport proteins, methionine is particularly reactive with ROS and RNS, including hydrogen peroxide, free hydroxyl radicals, hypochlorite, chloramines, and peroxynitrate. MSR reduces surface methionine sulfoxide residues without the necessity of protein breakdown, allowing continued function of the respiratory chain membrane in lieu of ubiquitization and proteasome degradation. Preservation of cytochrome C in the face of methionine oxidation may avert its release from mitochondria, thus preventing the cellular internal pathway toward apoptosis. Apoptosis (self-induced cell death) of outer hair cells appears to be the chief mechanism of permanent hearing loss from excessive noise exposure.

Evidence is accumulating that methionine plays a key role in epigenetic signaling as well. S-adenosyl methionine either can lead to increased GSH synthesis for control and elimination of free radicals or can donate a methyl group for epigenetic methylation of cytosine residues in nuclear DNA. Intracellular concentration of GSH is exquisitely controlled at approximately 5 mM. GSH depletion leads adenosyl methionine to take the pathway through demethylation, to replenish GSH. Use of methionine for GSH production results in fewer pyrimidines for DNA production and diminished DNA cytosine methylation. Experimentally increasing intracellular methionine reverses this finding.

Thus, N-acetylcysteine and DMET work in different areas of the cellular redox system. We have experimentally demonstrated significant recovery of hearing following continuous noise exposure with treatment of low-dose DMET/NAC as well as DMET only, NAC appeared to delay recovery during the first 2 weeks in the DMET/NAC arm, which may indicate some negative interactive effect initially; DMET/NAC ultimately revealed only slightly better efficacy than DMET only. Although the DMET-only group showed faster improvement through week 2 than did the DMET/NAC group, the DMET/NAC group seemed to “catch up” at 3 weeks and ultimately showed only minimally better efficacy. Although DMET/NAC may have had some effect other than DMET only, this dose of NAC may have been too low to see a difference.

Our observations support the notion that at low doses, the combination of DMET and NAC administered together had no immediate effect, that is, did not prevent a temporary threshold shift. In fact, initially, we observed that all of the treated animals showed hearing farther from baseline than did the control group at many frequencies. We would note once again that the dose of NAC given was only one-fourth of the dose that has been shown to be even minimally effective and is less than 5% of the optimal dose of 325 mg/kg; the DMET dose is less than 10% of that used in previous studies and provided almost equal efficacy as higher doses.

Other studies have shown more effect with 200 mg/kg DMET, albeit less effect than with 400 mg/kg. Bielefeld et al reported an improvement with 50 mg/kg NAC administered intraperitoneally; 100 mg/kg was equally effective, although 325 mg/kg administered intraperitoneally led to more improvement. In this study, we were able to lower the NAC dose a full 4-fold and DMET to less than one-tenth the dose of previous studies and still restore hearing after acoustic trauma. It is possible that a slightly higher dose of NAC would have given better end results, and further animal studies are needed to ascertain the optimal dose in combination with DMET. Although the low effective dose of DMET may demonstrate more potency than NAC at the same dose, it does not imply that it is more efficacious. Alternatively, it may be that NAC and DMET actually compete for the same enzymes, and thus there is no increased benefit to the combination of the two.

As a comparison, in acetaminophen overdose, the dose of NAC is a total of 300 mg/kg over 24 hours and then 150 mg/kg per day for the next 48 hours, equivalent to 42 g for a 70-kg person. DMET, used for the same indication, is given as 10 g over 12 hours. Two points are important for relative dosing. First, cochlear damage caused by ROS and RNS production as a result of acoustic trauma may or may not compare with hepatic levels of free radicals in acetaminophen toxicity, and the ultimate effective dose required may be related to the amount of ROS and RNS produced as a result of either noise or acetaminophen overdose. Second, liver function studies are used as a biomarker of hepatic status during initial damage and to guide dosing. Currently, there is no biomarker to guide treatment for noise-induced outer hair cell damage.

Lower dosing may eliminate some side effects and make these compounds more palatable for long-term use in chronic noise. Oral NAC has been described as having “a putrid odor with its taste very difficult to mask” (p. 1826). Patients develop nausea and vomiting after the administration of oral acetylcysteine. Current oral dosing requires large pills; gastric uptake is reported to be 4%. NAC administered intravenously in conjunction with subtoxic acetaminophen levels in massive doses has led to pediatric status epilepticus, intracranial hypertension, and death. Bronchospasm and hypotension as an anaphylactoid reaction have been reported in up to 26.7% of patients with intravenous dosing. Oral dosing has rarely been associated with angioedema but is more commonly associated with nausea and vomiting. In animals, elevated liver enzymes and animal growth retardation occur when NAC is given in excessive amounts for chronic use. In contrast, several human studies that included long-term dosing up to 1 year with 1800 mg/d for idiopathic pulmonary fibrosis and an HIV study using 8 g/d for up to 24 weeks showed no adverse side effects. Both NAC and DMET have a track record of more than 20 years in the successful treatment of acetaminophen overdose. Once again, these studies may indicate that the safe dose is related to the quantitative free radical production, whether it is hepatic, pulmonary, or...
acoustic, and a biomarker for damaging free radical production could guide treatment.

In long-term animal studies, restriction of methionine increases life span. Methionine in high doses has previously been implicated as the cause of elevated blood homocysteine levels, possibly related to cardiac and cerebrovascular disease, type 2 diabetes, depressed growth rate, decreased longevity, neuropsychiatric disorders, neurodegenerative disorders including Alzheimer disease, and cancer. Recent studies in Alzheimer and vascular disease, however, have confirmed that folate added to a diet including methionine eliminates the methionine–homocysteine connection and that hyperhomocysteinemia in humans is less related to methionine level than to age.

Although this combination of DMET/NAC prevents permanent hearing loss from acoustic trauma, DMET appears to be the primary compound responsible for hearing restoration in the low dose administered. Considerations for efficacy and safety led us to evaluate the combination of DMET and N-acetyl-L-cysteine (NAC) paralleled control values and established significant recovery compared with controls only at 3 weeks. NAC-only animals showed no difference from controls after 3 weeks. It is possible that 12.5 mg/kg NAC is below any effective dose.

Figure 2. Decibels from prenoise baseline at (a) 2, (b) 4, (c) 6, and (d) 8 kHz with comparisons for all treatments and controls. Bars indicate standard deviation; *P < .05, **P < .001. Although animals receiving D-methionine (DMET) only recovered through the first 2 weeks and demonstrated minimal return toward baseline at 3 weeks, animals receiving both DMET and N-acetyl-L-cysteine (NAC) paralleled control values and established significant recovery compared with controls only at 3 weeks. NAC-only animals showed no difference from controls after 3 weeks. It is possible that 12.5 mg/kg NAC is below any effective dose.
Cochlear antioxidant levels, and it is unclear whether there might be a correlation between blood levels of the antioxidants, blood distribution to individual organs, and cochlear levels. Further studies would help to elucidate the effect of NAC in the mixture and establish combination dosing that is safe and efficacious for long-term use.

Disclaimer

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Departments of the Navy or Army, the Department of Defense, or the United States Government.

Author Contributions

Royce E. Clifford, analysis and interpretation of data, drafting and revision; John K. M. Coleman, concept and design, acquisition of data, revision; Ben J. Balough, concept and design, revising, final approval; Jianzhong Liu, concept and design, acquisition of data; Richard D. Kopke, concept and design, analysis and interpretation; Ronald L. Jackson, concept and design, analysis and interpretation, revision.

Disclosures

Competing interests: Richard Kopke is co-founder and officer of Otoloplastic Pharmaceuticals.

Sponsorships: None.

Funding source: Office of Naval Research, grant N0001401WR20207.

References


